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SOME AMERICAN SPECIES OF MARSILEA WITH SPECIAL REFERENCE TO THEIR EPIDERMAL AND SORAL CHARACTERS

K. M. GUPTA, D.Sc.

In connection with the monographic study of the genus *Marsilea* in India that has been undertaken in my laboratory at Jaswant College, Jodhpur, a loan of about one hundred and fifty sheets of *Marsilea* comprising eleven American species was received from the University of California Herbarium, Berkeley, for comparison with the Indian species. Of the species received, only two, *M. minuta* and *M. quadrifolia*, are represented in the Indian flora. Study of this material led to the paper here presented.

Marsilea is a genus of world-wide distribution. Just as *M. minuta* is of very wide occurrence in India from the Panjab in the north to Travancore in the south, *M. vestita* among the American species has a wide range of distribution throughout the western and Pacific United States and, like the Indian species, is very variable. It has been observed that *M. minuta* possesses varieties which are anatomically distinct, particularly in the internal organization of the sporocarps, but since this subject is now being studied by my research students from both the morphological and cytological point of view, I do not wish to anticipate their results herein. Miss Margaret Stason (1926), too, had hinted toward such a possibility while discussing the possible connection between *M. vestita* and *M. oligospora*. I should like to state that both these latter species seem distinct from one another.

Because the American material was limited in quantity, my study of these species is not as extensive as I would have liked. In some cases it was possible to remove only one or two sporocarps from the herbarium sheets for the study of their internal structures. However, the external characters were carefully studied and in spite of the fact that the genus is notorious for its morphological plasticity, one cannot always ignore certain differences like the shape of the leaflets (figs. 1–9) that might clearly be discernible among the allied species. I have, therefore, summarized these observations on the vegetative characters in Table 1.

On account of the limitations imposed for want of preserved material, I chose to remove only a few leaflets and some sporocarps from each of the collections in order to examine their epidermal and soral characters. It is a well known fact that with the publication of many important works on the epidermal and cuticular studies of vascular plants (Linsbauer, 1898; Porsch, 1905; Thomas and Bancroft, 1913; Rehfous, 1917; Bandulska, 1923, 1926; Prat, 1932; Florin, 1931 and afterwards; Allsopp, 1952, 1953a, 1953b, 1953c, 1954, 1955), the taxonomic importance of such studies has gained world-wide significance. Linsbauer was probably

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the first to study the epidermal characters in *Lycopodium*. Porsch had suggested the phylogenetic importance of stomatal characters, Rehfous thought that the stomatal apparatus exhibits a constant feature within a group, and according to Miller (1938, p. 322) "the structure of the stomatal apparatus is markedly different in different groups of plants." Prat, after his comprehensive work on the epidermal structure of the Gramineae, stated that epidermal structures and their distribution of parts are specific characters. He says (1932, p. 185), "Correctement interpretés, les caractères épidermiques peuvent être de même valeur que les caractères qui ont servi de base à la définition des groupes systématiques." Satake (1934) has shown the systematic importance of the epidermal elements in the leaves of Japanese selaginellas and has founded a new classification on the basis of the epidermal structure of the leaves. Chowdhury (1937) studied the epidermis of eleven Indian species of *Lycopodium* and found that characters of the epidermis in most of the species were of diagnostic value. Some study has been made in my laboratory on the structure of the epidermis of some Indian species of *Selaginella*. As far as I know no detailed investigation on the epidermis of *Marsilea*, a plant with pronounced amphibious habit, has been undertaken in the past. The present examination, though brief, is clearly indicative of its usefulness.

The soral characters in the sporocarps of the genus have been studied in the past, but their systematic value had not yet been fully recognized.

SPECIMENS EXAMINED. The University of California material studied was collected by various workers over a period extending from 1849 to 1954. Except for *M. vestita*, which has a very wide distribution in the United States, all of the specimens examined are cited below:

MARSILEA VESTITA Hook. & Grev. UNITED STATES. Western Texas to El Paso, New Mexico, *Wright 811*, May-October 1849. UTAH. Rich County: Bear Lake near Laketown, *Porter 6490*, 30 June 1954. CALIFORNIA. San Diego County: *Purpus*, May-October 1898.

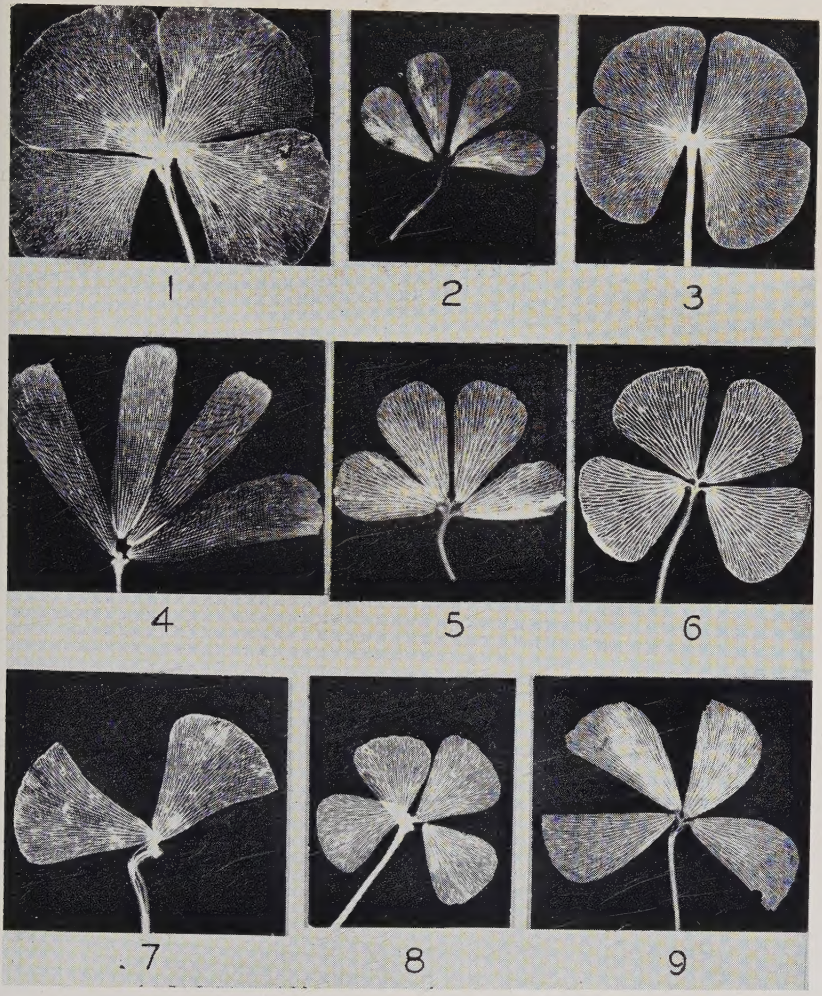
MARSILEA MOLLIS Rob. & Fern. MEXICO. CHIHUAHUA. St. Diego, *Hartman 604*, 20 April 1891. DURANGO. City of Durango and vicinity, *Palmer*, April-November 1896.

MARSILEA MEXICANA A. Br. MEXICO. CHIHUAHUA. Near Cusihuiriachic, *Pringle 2007*, 23 September 1888. SONORA. San Pedro, *Hartman 893*, 1894.

MARSILEA MACROPODA Engelm. MEXICO. TAMAULIPAS. Near Matamoras, *Pringle 1975*, 8 August 1888. UNITED STATES. TEXAS. (New Braunfels?), *Lindheimer 573*, 1846. Ponds on the Seco, *Reverchon 1630*, June, no year. Dimmit County: Turkey Creek south of Crystal City, *Muenscher & Winne 16506*, 29 June 1945. Jackson County: Lavaca River, *Tharp*, 29 August 1941.

MARSILEA FOURNIERI C. Chr. MEXICO. NUEVO LEON. Rio de San Juan outside of China, *Barkley 14344*, 27 February 1944. SONORA. Rancho San Carlos, 40 miles west of Hermosillo on road to Kino Bay, *Wiggins & Rollins 181*, 30 August 1941. BAJA CALIFORNIA. Seventeen miles south of Pozo Aleman, *Wiggins 7848*, 3 March 1935; 20 miles south of Calmalli, *Wiggins 5422*, 18 April 1931.

MARSILEA UNCINATA A. Br. UNITED STATES. Haven's Ranch, *Lemmon*, July 1882. TEXAS. Grown at Berlin Botanic Garden from fruit collected in Texas in 1872 by *E. Hall.* Comanche Spring, New Braunfels, *Lindheimer 1283*, June 1851. Caldwell County: dry sink, prairie, *Barkley 13130*, 7 July 1943.



Figs. 1–9. Photographs of the quadrifoliate leaflets of *Marsilea* species, rendered transparent so as to show exact outlines and venation. 1, *M.* cf. *macropoda,* × 0.8; 2, *M. minuta,* × 1.3; 3, *M. mucronata,* × 1.3; 4, *M. tenuifolia,* × 1.3; 5, *M. mollis,* × 2; 6, *M. vestita,* × 1.3; 7, *M. uncinata* (lacking two leaflets), × 2; 8, *M. oligospora,* × 1.3; 9, *M. fournieri,* × 1.8.

Marsilea tenuifolia Engelm. UNITED STATES. Texas. Inks Lake, *Tharp,* 11 August 1941. (New Braunfels?), *Lindheimer 745* in 1847. Llano County: creek near Kingsland, *Whitehouse 18480,* 4 May 1947; creek bed, *Tharp,* 15 August 1940.

Marsilea oligospora Goodd. UNITED STATES. Wyoming. Uinta County: Jackson Lake, *Nelson 6560,* 12 August 1899 (isotype). Elmore County: King Hill, *Nelson* & *Macbride 1158,* 17 July 1911. Sublette County: near New Fork Lake, *Payson* & *Payson 4437,* 24 July 1925; Kendall, *Payson* & *Payson 2920,* 5 August 1922.

Marsilea mucronata A. Br. UNITED STATES. North Dakota. Valley City, *Stevens 1223,* 1 August 1950. Benson County: Butte, *Lunell,* 5 September 1905. Nebraska. Exeter, *Wibbe,* September 1888. Wyoming. String Lake, *Prettyman,* 20 July 1953.

Marsilea quadrifolia L. UNITED STATES. Connecticut. New Haven, *Setchell,* 3 October 1883. Cromwell, *Hubbard 6817,* no date. Litchfield County: Bantam Lake, *Bridgman,* 1888, *Thompson,* August 1891. Massachusetts. Falmouth, *Brooks,* 30 August 1910. Salem, *Harper* & *Harper,* 19 July 1895. Glacialis Pond, Cambridge, *Pease 5672,* 9 October 1904. Norfolk County: Wellesley, *Brown,* 14 August 1940. Kentucky. Fayette County: two miles east of Lexington, *McFarland 46,* 25 September 1940.

Marsilea minuta L. MEXICO. Coahuila. Cerro de Cypriano, *Purpus 4525,* July 1910. Sinaloa. Culiacan, *Brandegee,* 11 November 1904. Baja California. San Jose del Cabo, *Brandegee,* September 1893, 16 October 1899, 25 October 1902. Jalisco. Near Guadalajara, *Pringle 2434,* 6 December 1889.

Marsilea cf. macropoda Engelm. MEXICO. Coahuila. Rancho Agua Bueno, 43 miles north of Monclova, *Gould 6405,* 20 June 1952.

In studying the above-cited specimens, the vegetative and reproductive structures were prepared in the following manner.

Hairs. The hairs from different regions (leaves, nodes, and the sporocarps) were removed dry and mounted directly in liquid paraffin on a clean slide in the manner usually employed for the examination of fibers of wool or cotton, so as to bring out their medullated or non-medullated nature quite distinctly.

Epidermis. For studying the epidermal structures, the leaves were treated with dilute KOH solution, washed in water, carefully teased, and stained with a single stain, safranin. After usual dehydration they were mounted in Canada balsam for examination.

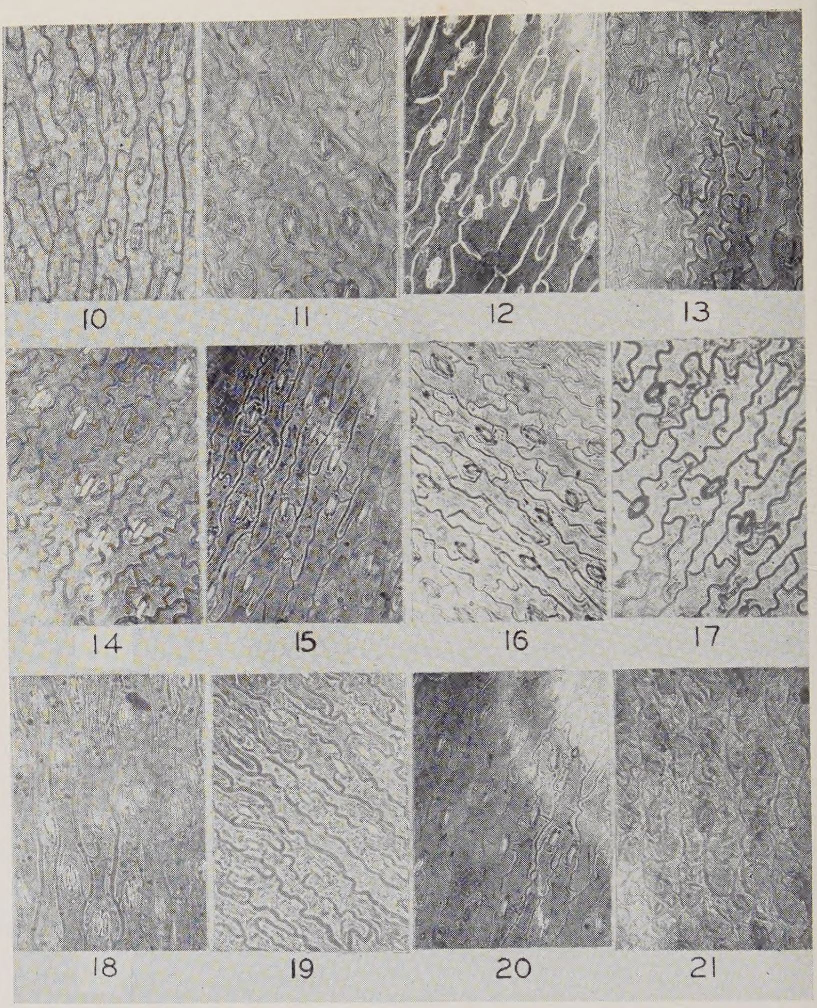
Sporocarps. The sporocarps were examined dry for their external features, but in order to ascertain their soral number, they were soaked in warm water after slightly scratching their resistant walls with a knife. This facilitated the so-called germination. The coming out of the mucilaginous mass is a mechanical process and cannot be strictly described as germination, as people often believe, because the real germination would mean the future activity of the mega- and microspores leading to the production of the gametophytes. The sporocarps were dissected further to study the nature of the sorus, the structure of the mega- and microsporangia, and also to find out whether the sporocarps were normal or possessed abnormalities which are often of a fluctuating type.

Range of Variation

The habitats of *Marsilea* vary from aquatic to subterrestrial or terrestrial; almost all species start as aquatic plants during which time their vegetative growth is pronounced. Some, like *M. minuta,* also produce sporocarps in the aquatic habitat, while others, like *M. aegyptiaca,* never do so. The majority of the species possess the tendency to fruit only under a subterrestrial habitat, and in fact most of the specimens from the University of California Herbarium seem to have been collected under subterrestrial conditions. They grow in plains (100 feet above sea level) as

TABLE 1. VEGETATIVE CHARACTERS OF THE NORTH AMERICAN SPECIES OF MARSILEA

General							Hairs			Epidermis		
Name of species	Habit	Length of petiole			Shape of leaflets	A/B Leaflet ratio	Average length in mm. and number of cells			Stomatal frequency	Size of stomata	Wall of epidermal cells
		Min.	Max.	Ave.			nodes	leaves	sporocarps			
M. vestita	Aquatic, sub-terrestrial and terrestrial	1.5	5.5	3.4	Obovate	0.33 : 0.29	1.7; 7	1.4; 4	1.4; 7	22	0.02 x 0.01	Long, somewhat straight
M. mollis	Not mentioned	0.5	1.1	0.84	Cuneate	0.18 : 0.08	-	1.0; 4	1.8; 5	10	0.03 x 0.02	Highly wavy
M. mexicana	Subterrestrial	5.0	6.3	5.6	Cuneate	0.33 : 0.32	-	-	-	8	0.02 x 0.01	Less wavy
M. macropoda	Aquatic and subterrestrial	1.4	8.3	4.5	Obovate	0.39 : 0.35	1.3; 9	1.9; 6	2.0; 9	9	0.03 x 0.01	Long and smooth
M. fournieri	Subterrestrial and terrestrial	1.0	2.4	2.69	Obovate	0.2 : 0.14	2.9; 8	1.6; 5	1.3; 6	17	0.02 x 0.02	Long, narrow,wavy
M. uncinata	Terrestrial	1.1	3.0	2.45	Cuneate	0.24 : 0.22	1.7; 7	0.5; 3	0.5; 5	13	0.02 x 0.01	Wavy
M. tenuifolia	Aquatic	2.5	6.3	3.79	Oblanceolate	0.94 : 0.4	1.5; 10	0.6; 3	1.0; 5	6	0.03 x 0.01	Straight
M. oligospora	Aquatic and terrestrial	0.4	2.2	1.3	Obovate	0.24 : 0.18	1.8; 8	1.1; 4	1.2; 6	6	0.02 x 0.01	Wavy
M. mucronata	Terrestrial	1.2	4.5	2.91	Cuneate	0.31 : 0.23	0.3; 7	0.5; 3	0.5; 3	5	0.03 x 0.02	Wavy
M. quadrifolia	Aquatic, sub-terrestrial and terrestrial	2.6	11.1	4.34	Obovate	0.45 : 0.41	1.5; 6		1.8; 6	5	0.02 x 0.01	Straight
M. minuta	Subterrestrial	1.5	6.0	3.4	Cuneate	0.29 : 0.18	1.16; 7	0.9; 4	-	17	0.02 x 0.01	Wavy
M. cf. macropoda	Subterrestrial	10.6	14.0	12.6	Fan-shaped	1.04 : 1.11	1.3; 8	2.0; 6	1.6; 6	8	0.02 x 0.01	Wavy



FIGS. 10–21. Photomicrographs of the epidermis of American Marsileas, showing nature of the epidermal cells and distribution of stomata. 10, *M. vestita*; 11, *M. oligospora*; 12, *M. macropoda*; 13, *M.* cf. *macropoda*; 14, *M. mollis*; 15, *M. mexicana*; 16, *M. fournieri*; 17, *M. uncinata*; 18, *M. tenuifolia*; 19, *M. minuta*; 20, *M. mucronata*; 21, *M. quadrifolia*. All about × 85.

well as on mountains, 6000 feet or above. Not only this, but some species like *M. vestita* are found in the plains (160 feet above sea level) as well as in the higher altitudes (5800 feet), whereas a species like *M. mollis* is confined to higher levels (6000 feet) only.

The rhizome is creeping and branches dichotomously. The internodes are short or long, sometimes becoming very much reduced. The adventitious roots, few or more in number in different species (one to eight), usually arise ventrally from the nodes of the rhizome. The size of the petiole varies considerably (from one to nine inches in length in different species), and so also the shape and size of the leaflets. The oblanceolate leaflets of *M. tenuifolia* (fig. 4) are quite peculiar to the species (Table 1).

The detailed examination of the structure of the hairs revealed the differences that exist among them in the different organs in the same species or on the same organs of the different species. The main point of interest is centered around the shape and size of either the basal or the terminal cells of the hairs of these organs (Table 1).

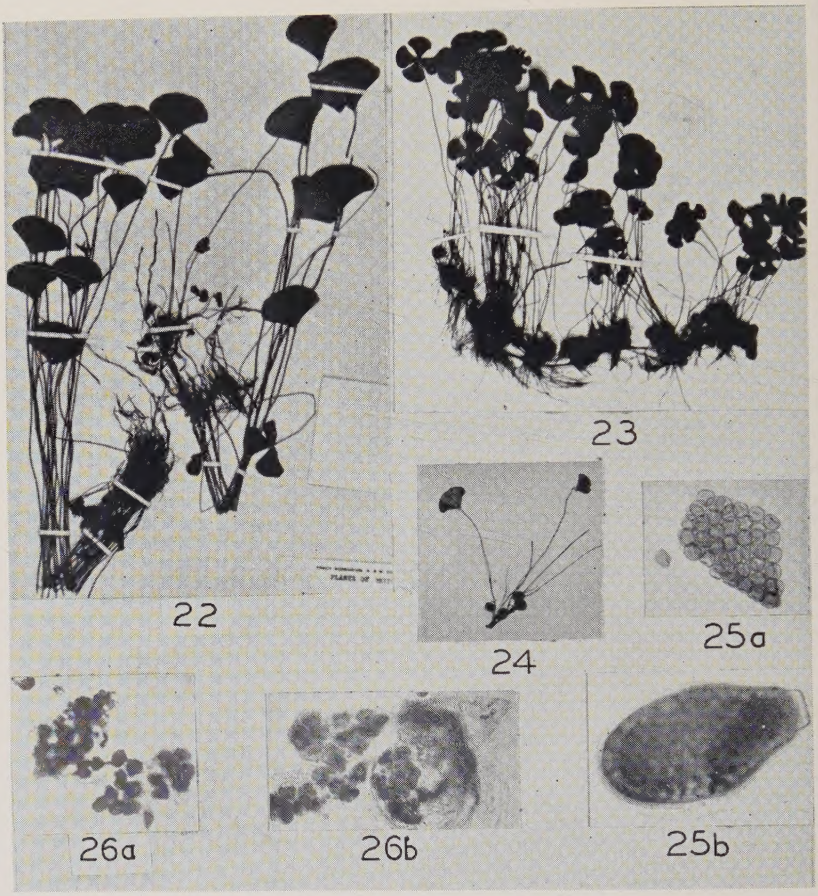
The study of epidermal structures has revealed that the species seem to differ from one another at least in two important respects, namely nature of the epidermal cells and the frequency of the stomatal distribution. The walls of the epidermal cells are either smooth, slightly wavy, or highly so, and the frequency of the stomata per unit area ranges from five to twenty (Table 1).

The sporocarps are stalked. Their attachment to the petiole is either basal or adnate, and the number of the sporocarps in a group may be one or more, connate or free (figs. 27–38). The shape differs from species to species, for instance it is subspherical in *M. minuta* (fig. 37), oval in *M. mollis* (fig. 28) and clearly bean-shaped in *M. vestita* (fig. 27). All the American species possess two horns, these varying in their prominence and bluntness. Similarly there is a difference in their soral number, a fact that seems to be of some systematic importance in the genus *Marsilea.*

Discussion of the Species

Marsilea vestita and M. oligospora. *Marsilea vestita* is very well represented in the collection by 84 sheets which date from 1894 to 1954. While the general characters are given in tables 1 and 2, I should like to mention a few interesting observations on this species. The anatomy of the epidermis shows that the walls of the epidermal cells are long and more or less straight (fig. 10). The stomata are uniformly distributed throughout, their orientation being in the direction of the length of the epidermal cells. The frequency of the stomatal distribution is almost the highest among the species here examined, namely twenty-two per unit area.

There is a single stalked sporocarp in *M. vestita* which is attached at the base of the petiole. It is bean-shaped and possesses both the horns, the upper pointed and the lower one blunt (fig. 27). An abnormal specimen with a sporocarp adnate to the petiole was found on sheet UC 205105 (fig. 24). Most of the specimens possessed normal sporocarps with normal type of both mega- and microspores (figs. 25a, 25b), but a critical search of the specimens revealed the presence of abnormal sporocarps (fig. 26a). The latter had no megaspores but only microspores. Sometimes these microspores were also of two types (fig. 26b). As pointed out in the



FIGS. 22–26. FIG. 22, *M.* cf. *macropoda,* habit showing largest leaflets and petioles, ca. × 0.25. FIG. 23, *M. macropoda,* same for comparison, ca. × 0.25. FIG. 24, *M. vestita,* abnormal specimen found on UC 205105, sporocarp adnate to petiole, × 0.25. FIG. 25, *M. vestita,* megaspores (25b) and microspores (25a) from a normal sporocarp, × 25. FIG. 26, *M. vestita,* microspores from two different sporocarps (26a, abnormal; 26b, normal), × 25. Generally in abnormal sporocarps megaspores are absent.

introduction, such a thing was expected in a species with a wide distribution; however, the significance of these and such other abnormal sporocarps as have been discovered in more than one Indian species is not discussed here.

It is important to point out that Miss Stason's observation (1926, p. 478) that *M. oligospora* may be an "ecotype" of *M. vestita* may not be quite correct, for I find that the epidermal as well as soral characters are distinct in the two species (figs. 10, 11, 27, 34) insofar as they are represented in the collection studied. For these reasons I prefer to regard *M. oligospora* as a distinct species rather than just an ecotype of *M. vestita.*

Marsilea macropoda and M. cf. macropoda. Five sheets in the series studied represent *M. macropoda* (fig. 23) and one specimen is questionably referred to that taxon as *M.* cf. *macropoda* (fig. 22). At the outset I should like to mention that these two look quite distinct from one another. The latter possesses not only larger leaves and sporocarps than *M. macropoda,* but probably larger than any species (figs. 1–9) represented in the series here treated. The epidermal cells in *M. macropoda* possess long and smooth walls (fig. 12), whereas in *M.* cf. *macropoda* the epidermal cells possess wavy cell walls (fig. 13) with stomatal frequency, however, being nearly the same in the two cases, namely nine and eight, respectively.

The sporocarps are stalked and tufted at the base of the petiole in *M. macropoda* (fig. 30). They are oval in shape and the biggest in size as compared with other species. On the other hand a group of only two or three sporocarps which are slightly adnate to the petiole is found in the specimens of *M.* cf. *macropoda* (fig. 38). The sporocarps in the two cases are, however, densely covered with hairs and possess both the horns, the lower one being more prominent.

One of the petioles in *M. macropoda* bears five leaflets instead of the usual four. This is not a very unusual feature in Marsileas, having been described for *M. quadrifolia* in 1948 and *M. aegyptiaca* in 1956.

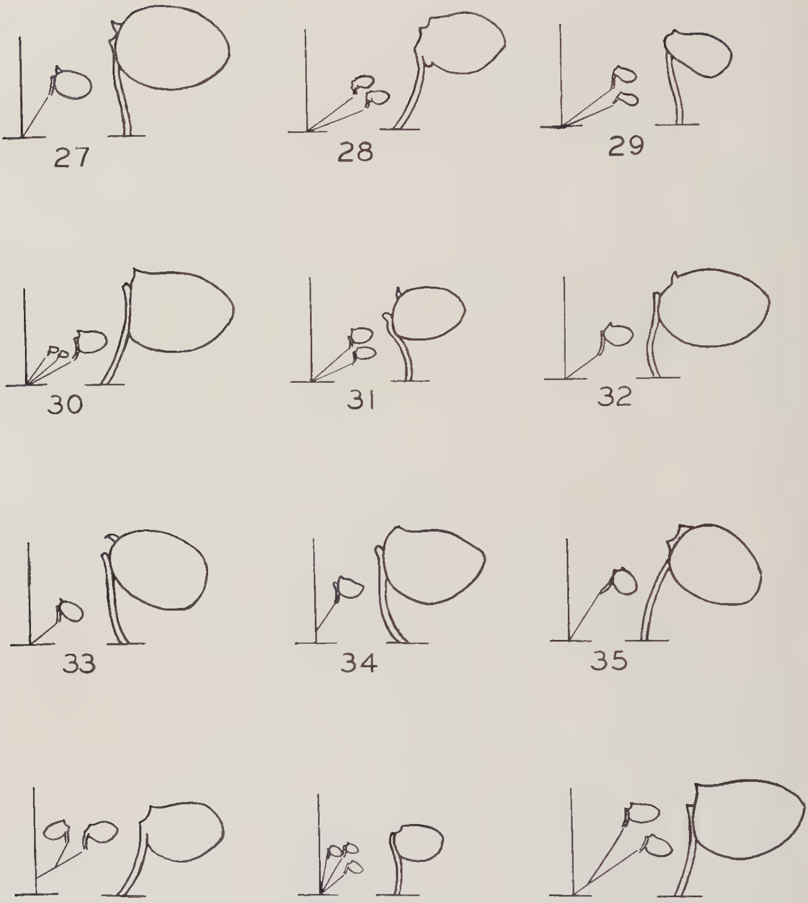
Marsilea mollis. There is only one herbarium sheet of this species, containing plants collected at two different times (1891 and 1896). The habit of this species is quite characteristic, with small hairy leaflets. There are no rhizomes or roots preserved; perhaps these are very much reduced. The epidermal cells of the leaves show wavy nature in their walls (fig. 14) and the uniformly distributed stomata show a frequency of about ten stomata per unit area.

The stalked sporocarps are tufted in nature, and their exact mode of attachment is not possible to describe in the absence of the rhizomes. The hairy and ribbed sporocarps are more or less oval in shape and possess two blunt horns (fig. 28). The soral number is about 15 in the species.

Marsilea mexicana. The two herbarium sheets of this species (collected in 1888 and 1894) are badly preserved. The leaves show epidermal cells which are less wavy and possess stomatal frequency of only eight per unit area (fig. 15).

The sporocarps are a bit characteristic in being somewhat narrower on the sides and swollen in the middle (fig. 29). They are ribbed and possess two blunt horns, with soral number twelve.

Marsilea fournieri. The four herbarium sheets contain material from both subterrestrial and terrestrial habitats. The leaves are obovate. The epidermal cells are long and narrow with their walls wavy in nature (fig. 16). The stomatal frequency is quite high, as much as 17 per unit area.



Figs. 27–38. Outline diagrams showing attachment of sporocarps to the petiole and the peduncles as well as the shape of the individual sporocarps. 27, *M. vestita*; 28, *M. mollis*; 29, *M. mexicana*; 30, *M. macropoda*; 31, *M. fournieri*; 32, *M. uncinata*; 33, *M. tenuifolia*; 34, *M. oligospora*; 35, *M. mucronata*; 36, *M. quadrifolia*; 37, *M. minuta*; 38, *M.* cf. *macropoda*. All about × 30.

The stalked sporocarps remain tufted at the base of the petiole. They are clearly bean-shaped and possess both the horns, which are pointed. The upper horn is further characterized by being longer and curved (fig. 31). The ripe mature sporocarps are quite smooth and ribbed, otherwise the species, like *M. macropoda,* is profusely covered with hairs.

Marsilea uncinata. In the four herbarium sheets the vegetative characters are quite variable, as for instance the number of roots (2 to 8) on the nodes and the size of the internodes. The leaves are cuneate, possessing epidermal cells with wavy walls (fig. 17). The stomatal frequency is thirteen per unit area.

There is a single stalked sporocarp which is oval in shape and is attached almost at the base of the petiole. There are two horns present on the sporocarp, the upper one being pointed and curved (fig. 32). The pedicel is fully adnate to the body of the sporocarp, which is smooth and possesses 16–18 sori.

MARSILEA TENUIFOLIA. The four herbarium sheets all contain material collected in an aquatic habitat. The leaves are very characteristic of the species, being oblanceolate in shape (fig. 4). The anatomy of the epidermis shows that the epidermal cells are long and narrow. The walls of the epidermal cells are smooth (fig. 18). The distance between the two walls of the same epidermal cell is narrow in the non-stomatal region and increases in the stomatal region as if to house the stomata. The frequency of stomatal distribution is 6 per unit area.

The single bean-shaped sporocarp is attached by a short stalk at the base of the petiole. There are two horns present; the upper one is pointed and curved downwards (fig. 33); the wall of the sporocarp is smooth and not hairy. It may be noted that there were only two sporocarps present on one specimen, while other plants were sterile.

MARSILEA MUCRONATA. The four herbarium sheets were all collected from a terrestrial habitat. The leaflets are cuneate in shape. The anatomy shows epidermal cells with wavy cell walls (fig. 20). The frequency of stomatal distribution is lowest in this species, being only five per unit area.

The sporocarps are usually solitary, rarely two arising from the base of the petiole. They are bean-shaped with the pedicels partially adnate to the sporocarps (fig. 35). There are two horns; both are pointed. The soral number is 16.

MARSILEA MINUTA and M. QUADRIFOLIA. Both these species are found widely distributed in India and are the common ones that have been described previously in and outside India (Pande, 1923; Mahabale & Gorji, 1948; Mehra, 1938; Puri & Garg, 1953; and Williams, 1920). And as a further study of the Indian species is in hand, it is not profitable to deal with them here, particularly in view of the fact that the University of California specimens did not contain enough material of well preserved nature.

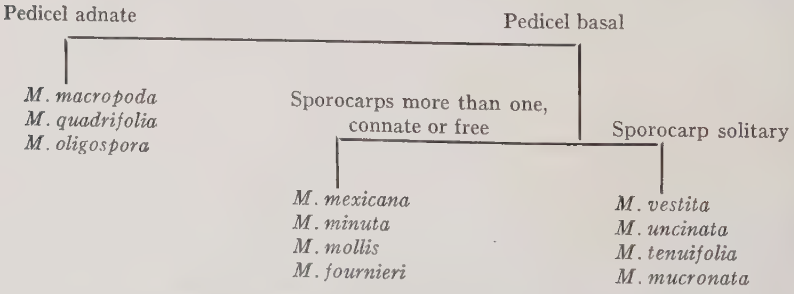
The epidermal studies (figs. 19, 21) in both the species have revealed that the stomatal frequencies in these are different, being five in *M. quadrifolia* and seventeen in *M. minuta*. Similarly the attachment of the sporocarp is characteristic in *M. quadrifolia*, being adnate to the petiole and connate to the pedicel (fig. 36), while in *M. minuta* two to six sporocarps are slightly connate at the base of the petiole (fig. 37). The comparison with the Indian material available in my laboratory indicated minor differences, but, in view of wide distribution of these species, these minor differences are natural.

Conclusion

From the foregoing brief description of eleven American species of *Marsilea,* it is apparent that the genus exhibits clear variations in the vegetative as well as in the reproductive organs, namely the sporocarps. In spite of a similar habitat, whether aquatic, subterrestrial or terrestrial, species do differ from one another, say for instance in the shape and size of their leaves (figs. 1–9) and structures of their sporocarps (figs. 27–38). The other types of variations that are often present in the same species under varied environmental conditions are distinctly different from the former differences, and are always exhibited within certain limits in that species. The latter should not be confused with the former. A thorough ecological study, therefore, becomes imperative, besides other important considerations of anatomy and cytology, not only to decide clearly the differences between two species but also to find out the range of variations within the species. Such an autecological aspect is being attempted with *M. aegyptiaca* here in the Botany Department at Jaswant College.

One simple aspect of morphology, namely the examination of the epidermis of the leaflets of these eleven species of *Marsilea,* has demonstrated how the structure of the walls of the epidermal cells and the frequency of stomatal distribution can be of some diagnostic value. It has been possible to indicate in the present thesis, for instance, that the species pairs *M. vestita–M. oligospora* and *M. macropoda–M.* cf. *macropoda* are quite distinct. If only the two above characters of the epidermis are taken into consideration, the present collection can be arranged into three different categories, namely, those with wavy walls, those with less wavy walls and those with smooth walls.

The structure and attachment of the sporocarps, however, provide the main clue to the correct identification of the species. The main features that are useful and essential for systematic considerations have been mentioned in Table 2. For the present, however, the number of sporocarps in a group and the nature of attachment of these sporocarps to the petiole have been adopted as a broad basis for classification (Gupta & Bhardwaja, 1956). Accordingly these American species can be arranged as follows:



It must, however, be admitted that the systematics of the genus *Marsilea* is a neglected subject, and it is hoped that as a result of detailed

TABLE 2. MORPHOLOGICAL CHARACTERS OF THE SPOROCARPS IN THE AMERICAN SPECIES OF MARSILEA

	External						Internal		
Name of species	Disposition	Relation of pedicel to petiole	Relation of pedicel to pedicel	Shape and size	Attachment of pedicel to sporocarp	Nature and number of horns	Soral number	Megasporangia and spores	Microsporangia and spores
M. vestita	Solitary	At the base	Free	0.18 Bean-shaped	Fully adnate	2, upper horn pointed	18	Normal or absent	Normal or abnormal
M. mollis	Tufted (?)	At the base	Not seen	0.11 Oval-shaped	Partially adnate	2, blunt	14-15	Normal	Normal
M. mexicana	Tufted	At the base	Partially connate, free (?)	0.11	Partially adnate	2, blunt	12	Normal	Normal
M. macropoda	Tufted	Slightly adnate at the base	Slightly connate at the base	0.25 Oval-shaped	Fully adnate	2, lower prominent	20	Normal	Normal
M. fournieri	Tufted (?)	At the base	Slightly connate at the base	0.11 Bean-shaped	Fully adnate	2, both pointed upper one slightly curved towards right	17	Normal	Normal
M. uncinata	Solitary	At the base	Free	0.13 Oval-shaped	Partially adnate	2, upper more pointed and more curved towards right	16-18	Normal	Normal
M. tenuifolia	Solitary	At the base	Free	0.13 Bean-shaped	Fully adnate	2, same as above	-	Normal	Normal
M. oligospora	Solitary rarely two	Slightly adnate at the base	Free	0.16 Oval-shaped	Partially adnate	2, lower more prominent, upper obscure	14	Normal	Normal
M. mucronata	Solitary rarely two	At the base	Free	0.2 Oval-shaped	Partially adnate	2, both pointed	16	Normal	Normal
M. quadrifolia	2, 3	Adnate	Connate	0.14 Oval-shaped	Fully adnate	2	16-20	Normal	Normal
M. minuta	2, 3	At the base	Free	0.11 Subspherical	Fully adnate	2	13	Normal	Normal
M. cf. macropoda	2, 3	Slightly adnate	Connate	0.21 Oval-shaped	Fully adnate	2, lower prominent			

investigation that is proceeding in the Botany Department at Jaswant College, some useful light will be thrown on the problem. Ecological, morphological and cytological studies of some Indian species seem to indicate a very promising field, and it may be possible in the near future to throw light on the nature of various so-called "ecotypes" possessing fertile or fluctuating type of sterile sporocarps, as a matter of fact, on the entire phenomenon of speciation in the genus *Marsilea*.

SUMMARY

Herbarium specimens representing eleven different species of *Marsilea* from North America have been examined, and in each case the structure of the epidermis and the sporocarps has been described. It has been shown that the species differ from each other in nature of their epidermal cells and the distribution of their stomata. So also they differ in external and internal characters of their sporocarps. The more important characters of the latter, as well as vegetative features in all these species, have been tabulated; in addition, the anatomical features of the leaves have been given in a series of photographs to indicate clearly that vegetative features, both external and internal, do contribute minor, if not major, criteria in identification of the various species of *Marsilea*.

ACKNOWLEDGMENTS

For the loan of the material of these American species of *Marsilea,* I am extremely grateful to Professor H. L. Mason, Director of the University of California Herbarium, and especially for his great kindness in allowing me to remove parts of the plants from the herbarium sheets for investigation, and for permitting me to keep the entire collection at Jodhpur for more than a year.

It is my pleasure to record the assistance given me by my two pupils, Shri T. N. Bhardwaja, M.Sc. and P. L. Mital, M.Sc., in preparing this paper for publication. My thanks are also due to my laboratory staff for their help in the preparation of the manuscript.

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CHROMOSOME COUNTS IN THE SECTION SIMIOLUS OF THE GENUS MIMULUS (SCROPHULARIACEAE). II.

Barid B. Mukherjee, Delbert Wiens, and Robert K. Vickery, Jr.

This cytological study[1], which is a continuation of a previous investigation of chromosome numbers in section *Simiolus* (Vickery, 1955), forms an integral part of a long range experimental study of the taxonomy, cytogenetics, and evolution of species in the genus *Mimulus* (Vickery, 1951). The counts were determined from observation of various stages of microsporogenesis. Many different techniques of fixation and staining were tried in an attempt to develop a better method than the one previously used (Vickery, 1955).

An effective and comparatively simple method was developed for obtaining chromosome counts. Buds of proper size, which varied from 1.6 to 3.1 millimeters, were killed and fixed for about two hours in a mixture of one part acetic acid to two parts distilled water. Buds that could not be studied immediately were stored in 70% ethanol. The best time for fixation proved to be from 9 to 11 a. m. The anthers were dissected out of the buds and stained in strong aceto-carmine. The stain was prepared by dissolving 1 gram of carmine in 100 mls. of boiling 45% acetic acid. It was cooled and filtered before use. The anthers were placed in a drop of stain on a microscope slide and heated gently over an alcohol flame. A cover slip was added and pressed firmly with a match stick to squash the anthers. From time to time during the next half hour more stain was added, more pressure applied and more heat used. The excess stain was removed with a paper towel pad. The coverglass was sealed with a half and half mixture of beeswax and paraffin. In a few cases propionic acid was substituted, with equally satisfactory results, for acetic acid in the above schedule.

The slides were examined within a day or two and the best figures found were drawn with the aid of a camera lucida (fig. 1). The method of Bhaduri and Ghosh (1954) was employed to make the slides permanent but with only moderate success. Herbarium specimens of all the cultures counted have been prepared for future reference and will be deposited in the Garrett Herbarium of the University of Utah under the culture numbers given in Table 1.

The chromosome numbers were found to be n=16 for *M. dentilobus* Rob. & Fern. from Chihuahua, Mexico, n=15 for *M. glabratus* var. *utahensis* Pennell from southern Utah, and n=14 for the ten cultures of *M. guttatus* DC. from California and northern Utah. These taxa appear to form an aneuploid sequence and a geographic series linking a group of North American taxa reported as n=14 (Campbell, 1950, and Vickery,

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[1] The work was supported by grants from the National Science Foundation and the University of Utah Research Fund. The authors wish to thank Drs. W. W. Newby and C. M. Woolf for their helpful criticisms of the manuscript.

Table 1. Chromosome Counts in Mimulus, Section Simiolus

n = 16	*M. dentilobus* Rob. & Fern.	Sierra Charro, Chihuahua, Mexico. *Gentry 8073* (5324).
n = 15	*M. glabratus* var. *utahensis* Pennell.	Fremont River, Bicknell, Wayne County, Utah, altitude 7100 feet, *Vickery 600* (5265).
n = 14	*M. guttatus* DC.	Mono Inn, Mono County, California, altitude 6420 feet, *Clausen 2043* (5015).
		Darwin Falls, Inyo County, California, altitude 2500 feet, U. C. Herbarium 696020 (5017).
		Mt. Diablo, Contra Costa County, California, altitude 1000 feet, *Stebbins 703* (5052).
		Mt. Oso, Stanislaus County, California, altitude 1000 feet, *Vickery 190* (5346).
		Bountiful, Salt Lake County, Utah, altitude 4800 feet, *Vickery 331* (5835).
		Mill Creek Canyon, Salt Lake County, Utah, altitude 5800 feet, *Vickery 335* (5840).
		Alta, Salt Lake County, Utah, altitude 8800 feet, *Vickery 336* (5845).
		Kimball Junction, Summit County, Utah, altitude 6600 feet, *Vickery 341* (5856).
		Hailstone, Wasatch County, Utah, altitude 6300 feet, *Vickery 342* (5857).
		Rock Creek, below Davies Resort, Duchesne County, Utah, altitude 7600 feet, *Del Wiens* 8/5/56 (5968).

1955) with a group of South American taxa reported as 2n=32, 48, and ca. 64 by Sugiura, Maude, and Brozek, respectively (Darlington and Wylie, 1955). However, the distinctive morphological characteristics of *M. dentilobus* (Grant, 1924) suggest to the authors that it is not a link in this aneuploid series and is not closely related to any of the North or South American species of *Mimulus*. Furthermore, interspecific crosses (Vickery, 1956) indicate that *M. dentilobus* is genetically isolated from all the other taxa of its section. Therefore, it appears to be an evolutionary sideshoot from the main group of *Simiolus* species. On the other hand, the morphology of *M. glabratus* HBK., which is the only species of the section common to North and South America, indicates relationships to both the North and South American groups of species. An n=14 culture of *M. glabratus* var. *utahensis* (5048) hybridizes with members of the various taxa of the two groups although the hybrids are nearly sterile (Vickery, 1956). Therefore, in view of the possible evolutionary role of *M. glabratus* as a connecting link between the North and South American taxa

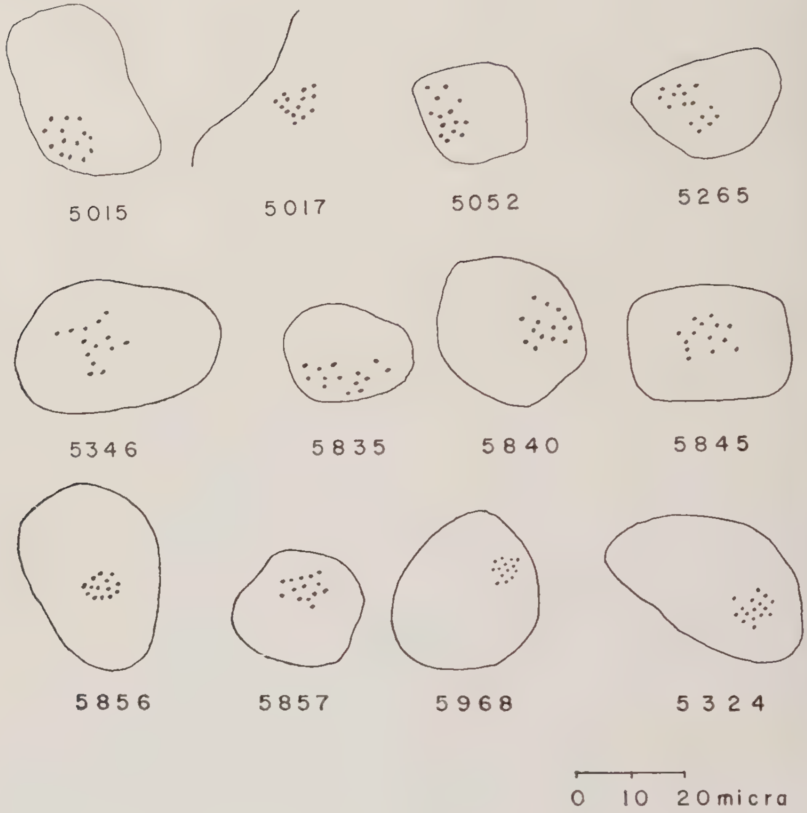

FIG. 1. Meiotic chromosomes of pollen mother cells of *Mimulus*, × 750. All drawings were made with the aid of a camera lucida. The numbers below the figures are the culture numbers (Table 1). The ten cultures of *M. guttatus* are n=14. The one culture of *M. glabratus* var. *utahensis* (5265) is n=15. *M. dentilobus* (5324) is n=16. All plants are in first metaphase except 5015, 5968, and 5324 which are in second metaphase.

of section *Simiolus*, the aneuploid number of n=15 for the Bicknell culture (5265) of *M. glabratus* var. *utahensis* is particularly interesting and significant. Further work is in progress to be sure that this count does not represent merely an aberrant individual.

The extensive hybridization experiments mentioned above (Vickery, 1956) reveal crossing barriers of various degrees between the different cultures of *M. guttatus*. However, in no case is a culture completely isolated from all the others. The results of these crosses suggested to the authors that all the cultures of the races of *M. guttatus* would have the same chromosome number. Our cytological observations confirm this idea and suggest, further, that the crossing barriers are due not only to gene differences but also to differences in chromosome structure. For example, culture 5968 has markedly smaller chromosomes than the other cultures. Probably there are cryptic structural differences in the chromosomes of the cultures as well.

In conclusion, we may report that our studies indicate that the North American *M. guttatus* complex of species ($n=14$) appears to be related to the South American *M. luteus* complex ($x=8$) by a series of aneuploid forms of *M. glabratus*. Work is in progress to determine the chromosome numbers of additional taxa and to determine the chromosomal homologies of the various cultures and races in order to clarify further our understanding of the evolutionary relationships in the group.

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ON THE SPECIFIC DISTINCTNESS OF RUDBECKIA LACINIATA AND R. AMPLA

GEORGE NEVILLE JONES

Wild goldenglow, *Rudbeckia laciniata* L. (Sp. Pl. 906, 1753), is a rather familiar plant growing in alluvial soil in eastern United States and adjacent Canada, ranging from Quebec to Manitoba and southward to eastern Texas and Florida. A morphologically similar plant described from Colorado in 1901 as *R. ampla* A. Nels., occurring in the western parts of the continent from Saskatchewan to South Dakota, New Mexico, Arizona, and Idaho, is less well known, and generally has been treated by contemporary students of the western flora as a synonym of *R. laciniata*.

There is evidence, however, on the basis of study of morphological characters, habitat, and habit, as well as geographical distribution, that these plants represent different taxa, apparently two distinct species. An alternative interpretation is that *R. ampla* might be treated as a "variety" or subspecies of *R. laciniata,* as the historical trend of human migration had led to discovery and publication of the latter taxon 148 years earlier. However, if one of them should be regarded as a subspecies of the other, the reverse process of designating *R. laciniata* as a subspecies of *R. ampla* is for certain reasons indicated as the more logical course.

According to a theory of origin of the genus *Rudbeckia* expressed by W. M. Sharp (Ann. Mo. Bot. Gard. 22:60. 1935), the present species are descended from an ancestral group originating on the Appalachian or the Ozarkian upland regions exposed since the close of the Paleozoic, the most recent of them inhabiting the Coastal Plain from Virginia to Texas. From this it would appear that *R. laciniata* and *R. ampla,* although morphologically similar, may have had different origins, the latter being much older, and the former of relatively recent origin on or near the Coastal Plain. It would be illogical to treat *R. ampla* as a subspecies of *R. laciniata* simply because the latter was discovered first. It seems therefore most practical to continue to designate each of them by a binary name. This procedure serves to express relationship satisfactorily, and there is no need for "new combinations" or other nomenclatural change.

Some of the diagnostic characters of these two species are summarized in the following key:

Heads ("disk") 1.5–2 cm. in diameter, becoming ellipsoid and 3–4 cm. in length; rays 9–15 mm. wide; disk corollas 5 mm. long; achenes 5–6 mm. long; receptacular bracts ("chaff") linear, 6–7 mm. long; pappus coroniform, the teeth short, obtuse, indistinct or none; leaves glabrous beneath, the upper surface somewhat strigose toward the apex of the leaf, the trichomes with enlarged pustular bases; basal leaves palmately ternate with broad segments and narrow sinuses; plants 1–1.5 m. tall; peduncles few, stout (1.5–3 mm. thick below the head)....*R. ampla*

Heads ("disk") globose, 1–1.5 cm. in diameter; rays usually less than 1 cm. wide; disk corollas 3–4 mm. long; achenes 3–4 mm. long; receptacular bracts spatulate, 3–4 mm. long; pappus crown of 4 teeth; upper leaves glabrous above, usually finely strigose beneath varying to nearly glabrous; basal and lower cauline leaves pinnately divided into relatively narrow segments with wide sinuses; plants 1.5–3 m. tall; peduncles several, slender (about 1 mm. thick below the head) *R. laciniata*

The relevant bibliographical references to *R. ampla* follow. It is not necessary to list here the extensive bibliography of *R. laciniata* L.

Rudbeckia ampla A. Nelson, Bull. Torrey Club 28: 234, 1901; Rydberg, Fl. Colorado 371, 1906, Fl. Rocky Mts., 927, 1917, Fl. Pr. & Pl. 836, 1932. *Rudbeckia laciniata* sensu A. Gray, Syn. Fl. 1(2): 262, 1878, ex parte; A. Nelson, Man. Bot. Rocky Mts. 544, 1909; Kearney & Peebles, Flowering Pl. Arizona 950, 1942, Arizona Flora 898, 1951; Davis, Fl. Idaho 777, 1952; Weber, Handb. Pl. Colorado Front Range 194, 1953; Harrington, Man. Pl. Colorado 598, 1954; non L., 1753.

Type locality. Colorado. The first cited specimen is *Baker 699* from Pagosa Springs.

To those who may be familiar with these plants the habit and appearance of members of the two taxa are noticeably different, although, as often happens in descriptive taxonomy, some of the differences are not easily stated in objective terms. In general, however, *Rudbeckia ampla* is a shorter, somewhat stouter plant with larger heads on fewer and stouter peduncles, the rays averaging somewhat wider. The disk corollas, achenes, and receptacular bracts are longer, the latter, commonly known as "chaff," furnishing distinctive characters that have been found to be important in the taxonomy of other species of the genus. Finally, it may be pointed out that the pappus of *R. ampla* is more coroniform, and the leaves are usually thicker and with different indument. In some specimens of *R. laciniata* the undersurface of the leaves tends to be less pubescent than in others, but almost all leaves show at least a few trichomes, particularly when viewed under the binocular microscope. It may be appropriate to note here that some published statements concerning *R. laciniata* are perhaps not as explicit as they should be. Unless modified, they may serve as a barrier to clarity of understanding of the plants described. For example, in the eighth edition of Gray's Manual the statements referring to the elongation of the "disk" of *R. laciniata,* as well as that indicating length of achenes, appear to be somewhat exaggerated, and are more nearly applicable to *R. ampla.*

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PINES FROM NUEVO LEON, MEXICO

BRUCE ZOBEL AND FRANKLIN CECH[1]

The forest geneticist usually attempts to grow as many as possible of the different strains and species of trees in which he is interested. These may be used for two purposes: 1) to establish a breeding arboretum, the trees to be used as parents for desired crosses; 2) to establish, on a limited scale, trials of adaptability to local or special environmental conditions. Both purposes were in mind when the Texas Forest Service sponsored two pine collection trips to the state of Nuevo León, Mexico. In conjunction with drought resistance studies, species that can do well under severe conditions of heat and drought were especially sought.

The accrual of further knowledge of the taxonomic and evolutionary position of the Mexican pines was another objective of importance. The purpose of this paper is to report on the several pine species and their many intergrading forms found growing in one of the states of northeastern Mexico, Nuevo León. Although collections were not made at all points in the state, the three areas visited were intensively studied, and

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[1] Silviculturist and Assistant Silviculturist, respectively, Texas Forest Service.

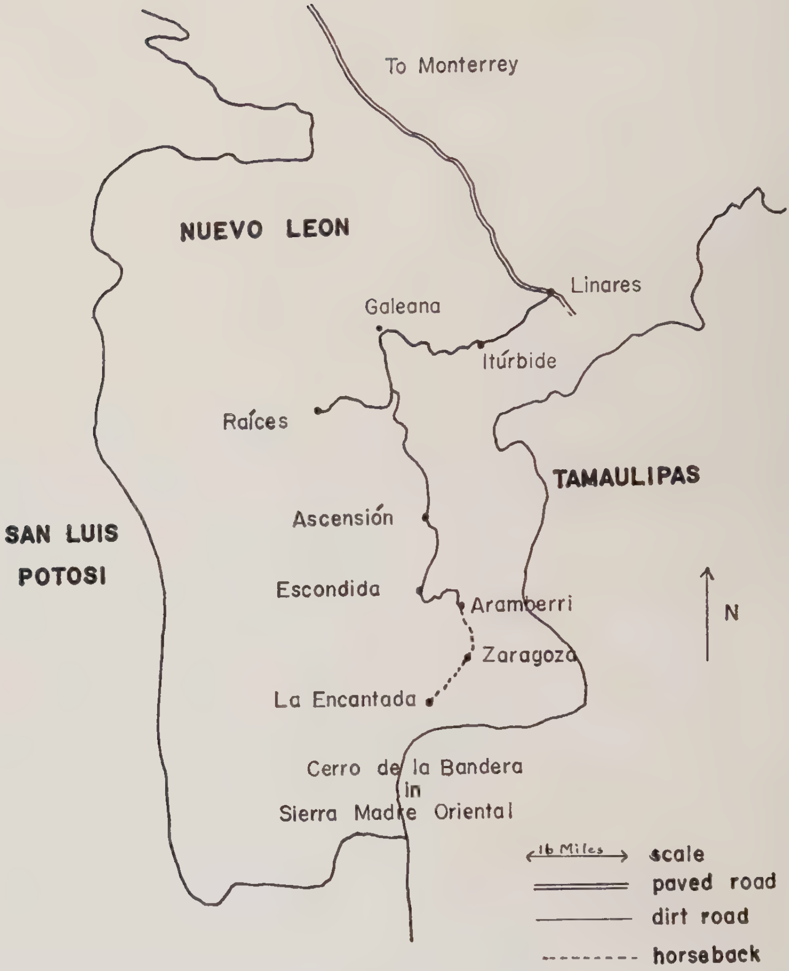


FIG. 1. Southern end of Nuevo León showing major area from which collections were made. The other two collecting areas were at more northerly points in the state.

complete records, specimens, and photographs were obtained for each species encountered.

The collection trips were the direct result of an invitation sent by Dr. E. A. Pequeño, Director, Instituto de Investigaciones Científicas, Universidad de Nuevo León. Though not a forester, Dr. Pequeño has an intense interest in the research upon and wise use of Mexico's forest resources, for he is fully cognizant of what their destructive exploitation will do to the economy of the country.

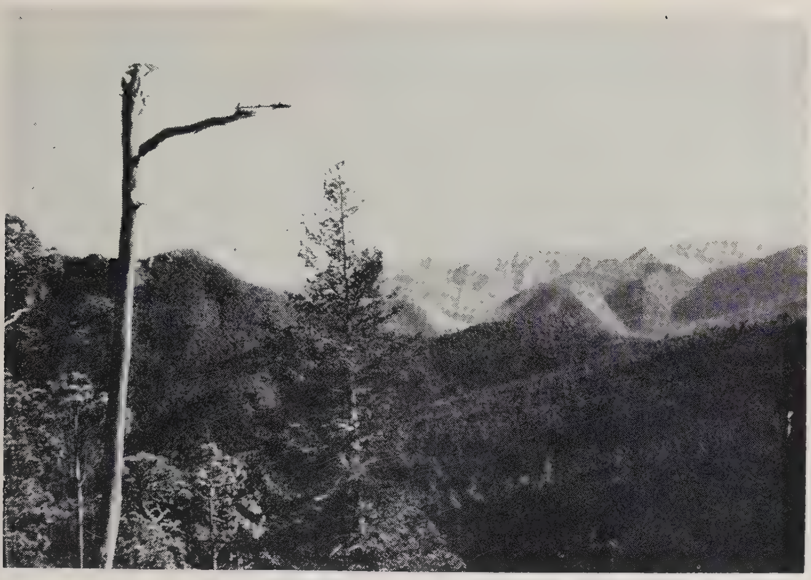


FIG. 2. Vicinity of La Encantada, illustrating the type and extent of the virgin forests in this area. A true fir (*Abies*) is in foreground.

The first trip was made in late September, 1954, with the senior author accompanied by Mr. Ruben Rocha, a Mexican student at Agricultural and Mechanical College of Texas, who acted as interpreter. This trip was preliminary in nature and little actual material was collected, because few pines bore cones that fall. However, many yearling cones indicated the possibility of a large cone crop for the following year. It was determined on this first trip that although a few species of pines matured their cones in September-October, the majority did not mature their cones until middle or late December or even in January.

Accordingly, the second trip was made late in December 1955. Both authors plus Mr. Chester Rowell, taxonomist from the Biology Department of the Texas A & M College and Mr. Rocha went on the second trip. The cone crop was plentiful on most species and abundant collections were possible.

The area from which most of the collections were made is in the extreme southern end of Nuevo León (fig. 1). As one goes into the mountains from Linares, the road which follows the stream bed rises sharply, going through the village of Itúrbide and then breaking out onto a plateau near Galeana. This unimproved road then proceeds in a southerly direction down the "plateau" through the towns of Ascensión, Aramberri, and (in 1954) to Zaragoza. From Zaragoza a trail leads up the mountain to the village of La Encantada, at an elevation of about 10,000 feet (fig. 2). Collections were made along this entire route wherever pines occurred,

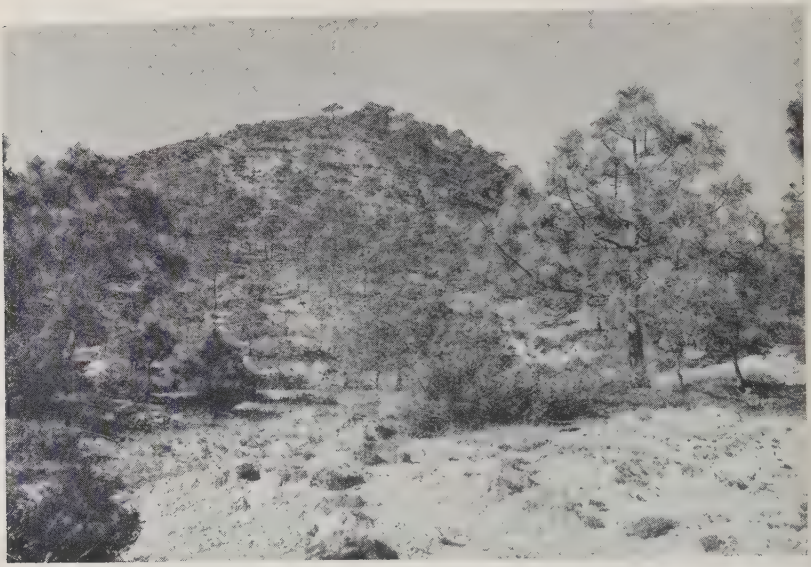


FIG. 3. *Pinus arizonica* var. *stormiae* on nearly solid rock in a very dry area near Ascensión.

most, however, being made up the mountain from Zaragoza and around La Encantada.

The collecting area described above varied from nearly desert near Galeana, Itúrbide, and Ascensión (fig. 3), with less than 20 inches rainfall, to the well-watered mountain forests around La Encantada. The higher elevation collections around La Encantada were in virgin forests, which included conifers of the genera *Abies* (fig. 2) *Pseudotsuga,* and *Taxus.* This is a most beautiful region, lush with vegetation. It is very similar in character to the middle-high elevations on the west side of the Sierra Nevada of California. Access is difficult, due to the poor roads and the necessity for horseback travel over considerable distances. This limited the specimens that could be collected. As far as could be determined, the pines around La Encantada had not previously been collected and classified, though Martínez (1948) mentions pine specimens from the area near Zaragoza.

Small collections were made from two other areas, one on Chipinque near the city of Monterrey, and the other in the Sierra Picachos near the village of Sombreretillo. This latter area is several miles to the east of the point where the Inter-American Highway from Laredo to Monterrey crosses the small range of mountains south of Sabinas Hidalgo.

All timbered regions at the lower elevations have been severely depleted. A crude method of bleeding for gum (oleoresin) is used extensively in the lowland areas, and in many cases so intensively and carelessly as to kill the trees (fig. 4). Fires and over-grazing have taken a huge toll and in

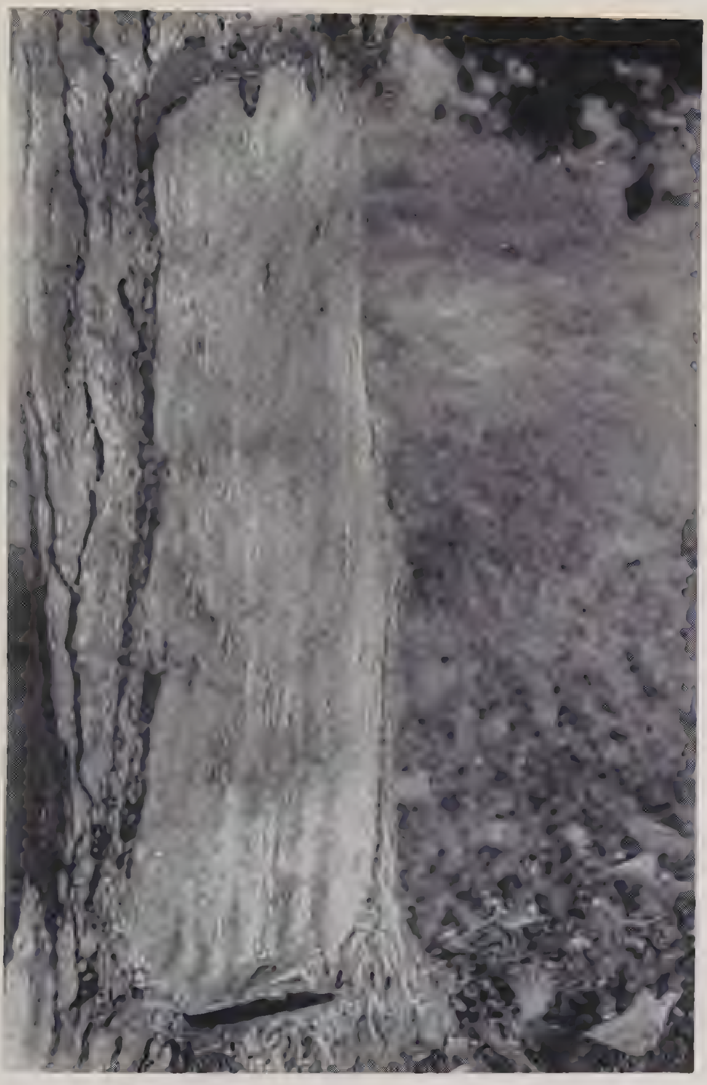


Fig. 4. Method of bleeding tree for gum (oleoresin). Note "cup" chopped in tree at base of peeled face.

many of the very scattered older stands, reproduction is lacking. There are indications in some areas that previously pine-covered land may degenerate to desert scrub if present treatment continues. Conversely the high mountain, virgin forests contain considerable overmature timber that is dying out. This could well be harvested if there were any method whereby the timber could be economically transported to markets.

In 1955, twenty-eight separate pine collections were made from the three areas visited. Each included: 1) ripe cones, up to a bushel from a single tree (these "green" cones were brought to Texas and the seed was extracted there); 2) a specimen fruiting branch, which contained mature cones, yearling cones, undamaged terminal bud, and typical foliage. These fruiting branches were pressed in the field; before this was done, however, a detailed study was made of their needles, buds, and other features, and colored photographs were taken to record foliage color, needle length and number, color and shape of unopened cone, color, position, and number of yearling cones, bud color, fuzziness, etc. In addition, needle bundles were preserved in formaldehyde-acetic acid-alcohol solution; these were later sectioned for the study of needle anatomy and morphology.

Current literature (Loock, 1950; Martínez, 1945 and 1948; Dallimore and Jackson, 1948) proved inadequate to the task of identification owing to the many recent changes in Mexican pine taxonomy and to the fact that the collections showed much intergradation. In order to make satisfactory species identifications of our collections, it thus became necessary to make a thorough analysis of all characters, including cross-sections of the needles, and then refer back to original sources in the literature of the genus.

Seedling Characteristics

In all books referred to, there is a notable absence of reference to seedling characters. Such characters are of importance, especially since many of the Mexican species show a tendency to have delayed height growth, i.e., a semi-grass stage of development. This tendency towards a grass stage of development might well be a key diagnostic feature, as is true for the intensively studied "southern pines" in the longleaf-loblolly-slash pine complex. A tree with a true grass stage grows one to many years putting on only very little height growth, the needles being borne near the ground and appearing as a clump of "grass." A tree with a semi-grass stage makes only very little height growth the first year, but some stem is evident; in subsequent years it may, or may not, put on rapid height growth. The seedlings of the material collected varied from nearly a true grass stage to normal height growth (fig. 5).

Seedlings of all collections in this study have gone through one growing season in the nursery. Their characters will be discussed in the species list which follows.

Pinus Species Collected in Nuevo León

Many species of pines, and their intergrades, were found in Nuevo León, with several species commonly growing completely intermixed.

Mexican pines do not display some of the usual differences held to be diagnostic of hard- or yellow-pines as contrasted with the soft- or white-pines. For example, many of the Mexican hard-pines contain five to seven or even more needles per fascicle, and five needles might be considered to be the rule rather than the exception. Conversely, the only true white-pine collected on this trip (*Pinus ayacahuite* var. *brachyptera* Shaw) had

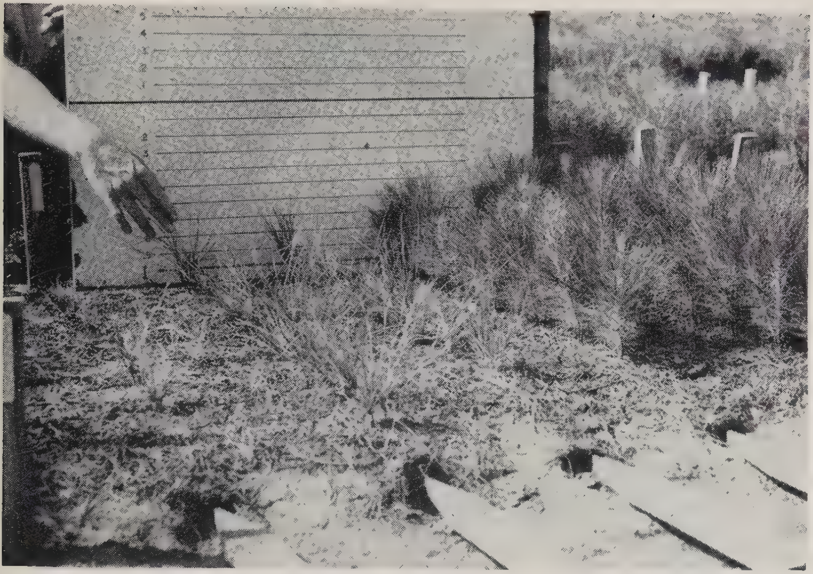


FIG. 5. Seedlings of *Pinus hartwegii* (on left) showing tendency toward a modified grass stage; seedlings of *P. teocote* (on right) showing normal growth in height and a basal crook in stem similar to shortleaf pine (*P. echinata*).

many fascicles containing only three needles instead of the normally expected five.

The five-needle hard-pines were most difficult to classify. Especially so were six specimens that exhibited characters of *P. montezumae* Lamb., *P. montezumae* var. *lindleyi* London, *P. pseudostrobus* var. *estevezi* Martínez, and *P. durangensis* forma *quinquefoliata* Martínez. Their characters did not fit any taxon completely, and after careful study the conclusion was drawn that they were intermediate or intergrading forms. Such intermediacy might well be called the rule here, especially for the five-needled pines. Both Loock (1950) and Martínez (1948) call attention to the intergrading forms and the complexity of the classification.

Four trees were found that had characters sufficiently dissimilar to previously described species so that they could not be definitely classified. These are included at the end of the species list. Three of the pine species in the list which follows have not been previously reported growing in the state of Nuevo León (Loock, 1950, and Martínez, 1948).

In addition to the pines, collections were also made of the genera *Abies*, *Pseudotsuga* and *Taxus*. The authors at first thought that *Taxus* had acquired a new distribution record, until it was found that Hernández *et al.* (1951) reported *Taxus* in the neighboring state of Tamaulipas. Many different oaks *(Quercus)* were observed, as well as other hardwoods such as Madroño *(Arbutus)*. This is an area rich in many forms of vegetation and an extended collection trip would be most worthwhile.

PINUS AYACAHUITE var. BRACHYPTERA Shaw. This species had intermediate characters, in many ways closely resembling *P. reflexa* Engelm. Needles were predominantly in fascicles of five, with many fascicles of 3, 4, and 6 being present on mature trees. Collections were made near La Encantada at an elevation of approximately 10,000 feet. This pine was growing among hard-pines, *Abies, Pseudotsuga,* and *Taxus*. The trees were very large on the better sites. Cones mature early (October and November) so no seeds were obtained.

PINUS ARIZONICA var. STORMIAE Martínez. Several collections of this species were made, from near Ascensión to the area around Galeana, at elevations from 5,000 to 6,000 feet. Classification was accurate. This species was growing on extremely rugged sites, with low rainfall. One collection was made on nearly pure calcareous soil, with little other vegetation being present. Seedlings, as well as mature trees, all had needles in fascicles of three. All seedlings had delayed height growth, a semi-grass stage of development.

PINUS CEMBROIDES Gord. Collections were made near Ascensión, at elevations around 5,000 feet; they were easily classified. This species was very widespread. Needles were mostly in fascicles of 2, a few fascicles having 3. Cones mature early and no seeds were collected.

PINUS HARTWEGII Lindl. Several collections were made of this species, most of which were easily classified. One collection had characters very similar to *P. rudis*. All collections were made near La Encantada, at elevations around 10,500 feet, in nearly pure stands. Needles were predominantly in fascicles of 5, some of 4. Seedlings all had their needles in fascicles of 3, and had a definite semi-grass stage (fig. 5). The seedlings looked somewhat like those of *P. pseudostrobus,* but were coarser.

PINUS PSEUDOSTROBUS Lindl. Two collections of this species were made, one fitting the classification well, the other having affinities with *P. hartwegii*. Collection was near La Encantada at elevations around 10,000 feet. These trees were growing in a mixed pine and fir stand, just below the pure *P. hartwegii*. Needles were mostly in fascicles of 5, with some of 6 and 7 present. Seedlings had needles in fascicles of 3, 4, and 5, and had a definite semi-grass stage similar to *P. hartwegii*.

PINUS PSEUDOSTROBUS var. ESTEVEZI Martínez. This species was collected only west of Itúrbide at an elevation of 5,000 feet. It was fairly easily classified, though all characters did not exactly fit. It was growing under relatively droughty conditions. Needles were in fascicles of 5, with some 4 and 6. Seedlings had needles in fascicles of 5. Unlike *P. pseudostrobus,* height growth of the seedlings was normal.

PINUS PSEUDOSTROBUS forma PROTUBERANS Martínez. It was difficult to classify this species accurately since no foliage was collected. Cones were collected at dusk from three trees, all appearing to be the same until cones were closely examined. Two of the trees remain unclassified, having needle anatomy like *P. hartwegii,* foliage like *P. rudis,* and cones similar to *P. pseudostrobus* var. *estevezi*. The third tree had cones exactly like *P.*

pseudostrobus forma *protuberans*. Collection was made near Ascensíon, at an elevation of 6,000 feet. Seedlings had a semi-grass stage, with needles in fascicles of 3, 4, and 5. This species has never been reported in Nuevo León, only from the more southern states.

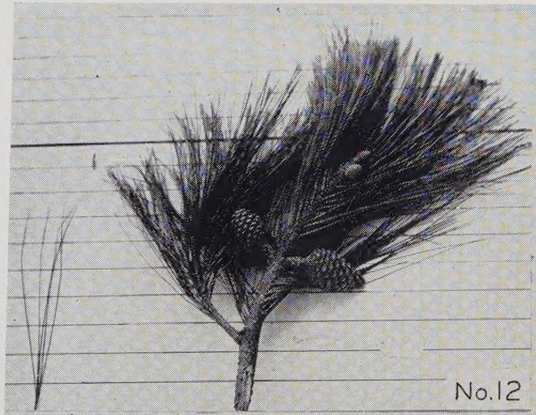
PINUS MONTEZUMAE Lamb. Several collections were made of this species, and classification was from very exact to doubtful. Two collections were very similar to *P. montezumae lindleyi* and also to *P. pseudostrobus*. Specimens were obtained near Zaragoza, Escondida, Ascensión, Itúrbide, and in the Sierra Picachos near Sombreretillo, ranging in elevation from 3,500 to 6,000 feet. Needles were in fascicles of 5, with an occasional 4. The needles of seedlings were in fascicles of 3, 4, and 5, the predominant number varying from tree to tree. Seedlings had extra long needles and had normal height growth (no grass stage). This was different from seedlings obtained from seed previously sent to us as *P. montezumae,* all of which had a pronounced grass stage.

PINUS MONTEZUMAE var. LINDLEYI Loudon. Classification of this species is only partially satisfactory, some characters not fitting too well. It was collected near Zaragoza at 5,000 feet elevation. Needles were in fascicles of 5, with some of 4. Seedlings had needles in fascicles of 5 and had normal height growth. This species has not previously been reported in Nuevo León, only from the states to the south, including Hidalgo, Vera Cruz, and Querétaro.

PINUS TEOCOTE Schl. and Cham. Of the several collections of this species, only one was in doubt, because of the needle anatomy. Collections were made near La Encantada, Ascensión, and at Chipinque, near Monterrey. Elevations ranged from 4,500 feet to 10,500 feet. Needles were short, always in fascicles of 3. Seedlings had all their needles in fascicles of 3, and had normal height growth (fig. 5). All had an unusual crook at the base of the stem, similar to that found in shortleaf pine (*P. echinata* Miller). Just as for *P. montezumae,* seed sent to us under the name of *P. teocote* had a grass stage, while none of our own collections showed this feature.

PINUS DURANGENSIS forma QUINQUEFOLIATA Martínez. Three collections were made of this species, none of which were classified with exactness. Characters were found similar to *P. pseudostrobus estevezi, P. montezumae* and *P. pseudostrobus*. This species has not previously been reported in Nuevo León, being found to the west in the states of Chihuahua and Durango. Our collections were made at La Encantada, Chipinque, and Sombreretillo. Needles were in fascicles of 5, with a few 4, and occasionally 6. Seedlings from two of the trees had needles in fascicles of 5, while one (from Sombreretillo) had them of 3, 4, and 5. Seedlings were tall (no grass stage), with long needles.

PINUS RUDIS Endl. One poorly classified collection was made of this species, the tree having characters like *P. montezumae lindleyi* and *P. pseudostrobus*. Collection was made at La Encantada, at 9,500 feet elevation. Needles were in fascicles of five. Seedlings were tall and had needles in fascicles of three, four, and five.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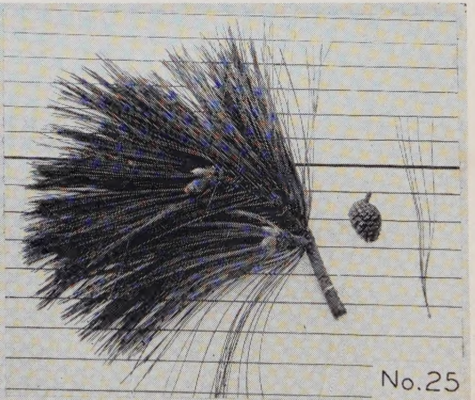
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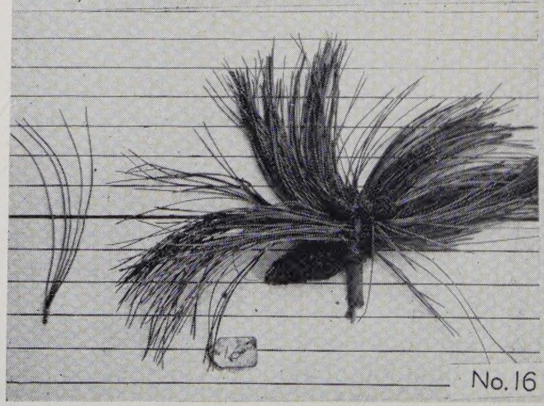
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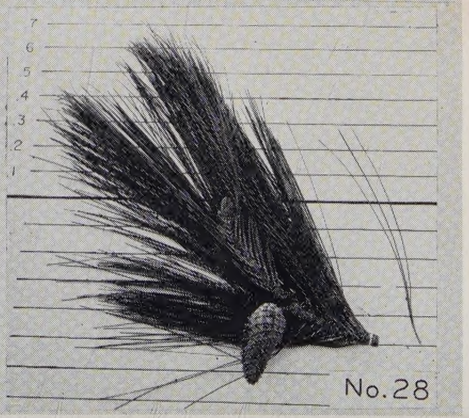
No. 28

The following *Pinus* collections from Nuevo León remain unclassified:

TREE NO. 12 (fig. 6). Growing on a very dry site near Ascensión, with cacti and a few xerophytic shrubs. Surprisingly vigorous for a tree growing on such a poor site. Foliage with needles all in fascicles of five, about 20 cm. long, thin, flexible, slightly drooping; resin ducts two to four; endodermal outer wall thickened; hypodermis with moderate intrusions into chlorenchyma; two vascular bundles touching but distinct. Cone 8 cm. long; peduncle about 2 cm. long; color greenish black when unopened, changing to dark brown with drying; apophysis strongly keeled, somewhat reflexed on dorsal part of cone; umbo very small, prickle not sharp, not deciduous. Seedling with needles in fives, thin; having normal height growth.

TREE NO. 16 (fig. 6). Collected only in one small valley above Zaragoza, growing intermixed with oaks at 6,000 feet elevation. Given by us the name "blue pine" due to the very bluish color of the foliage. Appearing to belong to the *P. rudis-P. hartwegii* complex, with intermediate characters. Foliage with needles predominantly in fives, about 18 cm. long, very stiff, twisted, glaucous blue-green; resin ducts seven to ten, medial; endodermal walls thin; hypodermis intruding slightly into chlorenchyma; two vascular bundles separated by several cell thicknesses of transfusion tissue. Cone about 10 cm. long, purplish black when unopened, olive brown when dried, asymmetric; apophysis flat to somewhat raised; umbo grey, sunken, very small. Seedling with needles in bundles of three, coarse; with modified grass stage.

TREE NO. 25 (fig. 6). Growing in creek bottom, very rocky, Sierra Picachos, near Sombreretillo. Foliage with needles in threes, fours, and fives, predominantly fives, about 23 cm. long, moderately heavy, drooping, blue-green; resin ducts three to six, medial; endodermal outer walls thickened; two vascular bundles close together, not distinct; hypodermis very thick with small intrusions. Cone (old, hanging on tree; no fresh cones) about 5 cm. long; peduncle about 1.5 cm. long; apophysis raised with flat face, slightly keeled; umbo flat to sunken. Seedling none.

TREE NO. 28 (fig. 6). From Sierra Picachos, near Sombreretillo. Needle number distinguishing this tree from *P. durangensis* and similar species. Foliage with needles predominantly in threes, few fours and fives, around 25 cm. long, somewhat pendent, moderately thin; resin ducts three and four, medial and one internal; endodermal outer walls variable, thick to slightly thick; two vascular bundles touching but distinct; hypodermis thick, with considerable intrusions. Cone about 9 cm. long, black to purplish green unopened, drying to light olive brown, somewhat asymmetrical; apophysis on dorsal side considerably reflexed, strongly keeled; umbo raised, ashy gray, with recurved, short, stout prickle. Seedling with needles in threes and fours, occasionally fives, thick; with normal height growth.

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